Patent Specification DE 43 34 677 C1
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Title: Culture Vessel with Observation Field for Microscopic Examinations

The invention relates to a culture vessel with observation field for microscopic examinations according to Fig. 1

Description

In the unexamined German application No. DE 34 46 908, an apparatus for the monitoring observation of microorganisms is described. The Utility Model No. DE-G 91 01 082.9 describes a means for examining the morphological state of biomasses in fermenters. An apparatus for the automatic observation of microorganisms in liquids with a video camera is described in the unexamined Japanese application No. 1-312992. These apparatuses and means are provided with electronic controls and are elaborate and expensive to manufacture. Furthermore, they are in direct connection with large plants, such as sedimentation tanks and fermenters. The unexamined Japanese application No. 1-317383 describes a container pair with observation window. This apparatus has to be turned upside down and sealed in order to be filled. The observation window is very restricted due to the container pair.

The invention disclosed below is a system located on a microscopic standard slide and can be easily and cheaply manufactured. Commercially available light-optical microscopes permit the use of the invention without any ancillaries or retrofitting. Moreover, light-microscopic properties are not affected as cover glasses having a thickness of 0.17 mm and being in correct flatness are used. For microscopic observation, a large field exists which can also be used for microscoping liquids with small particles in a flow that can be stopped at any time. Furthermore, there is the possibility of introducing liquids during microscoping, mixing them thoroughly or withdrawing them. The possibility of reusing the system after having it simply cleaned is another advantage.

The invention relates to a culture vessel with observation field for microscopic examinations. It is an object of the invention to provide a permanent habitat in a nutrient solution of approx. 1 ml for microorganisms, the habitat extending into an observation field (6) for microscopic

examinations with an appropriate depth of field. The microorganisms living in the observation field (6) have a connection (5) to the culture vessel (1) and can be studied over a long period without having to be removed from a vessel by pipettes. Microorganisms living at the interior wall of the observation field (6) can be unaffectedly observed, so that morphological examinations with microorganisms are possible over a relatively long period.

The invention can be microscoped in the path of light rays of any commercially available light-optical microscope with various illuminations (e. g. bright field, dark field, phase contrast). For strongly magnifying stereo magnifying glasses, the invention can also be used.

In a simple manner, microbiological phenomena can be studied with the invention. Application possibilities are to be hydrobiological laboratory examinations, microscopic control of flocculi of the activated sludge tanks in sewage treatment plants, limnological and marine plankton examinations, demonstrations with microscopes at trade fairs, permanent installations in botanical gardens, zoos or nature museums and connection to television and other video transmission and recording systems. Furthermore, the invention is also to be employed as teaching material or teaching toy. The invention is moreover to be easily and cheaply manufactured from glass or plastics.

This object is achieved by a culture vessel with observation field according to Fig. 1.

The culture vessel with observation field is manufactured by assembling two round rods or flat rods arranged in parallel on a slide with a cover glass by gluing thereby forming a permanent seal. The flat rods can be 0.25 mm thick strips of glass or plastics coated with an adhesive, or glass or plastic rods having a diameter of approx. 0.25 mm and being coated with an adhesive. The culture vessel consists of a threaded bottle the bottom of which is cut off and permanently glued to the slide and cover glass at the front side outside so as to provide a seal, the connection to the observation field remaining open inside.

Another possibility of manufacturing the culture vessel with observation field is to grind and polish a groove of a depth of approx. 0.25 mm into a slide. The observation field is formed by gluing the slide and a cover glass in a sealing and permanent manner. The front side of the culture vessel is subsequently permanently glued to the slide and cover glass so as to provide a sealing outside, so that the connection to the observation field remains open inside.

Another possibility of manufacturing a culture vessel with observation field is to grind open the bottom of a bottle just until a flat capillary can be introduced at a right angle to the standing bottle, which capillary is then glued to the bottle outside so as to provide a permanent seal, the connection to the observation field inside remaining open. Subsequently, the bottle can be temporarily or permanently mounted onto a slide, for example, by gluing. For the sealing and permanent connection in the above mentioned operations, adhesives should be used that achieve a high final strength with glass-like properties (resistance with respect to temperature, water, solvents, diluted acids and alkaline solutions as well as chemicals), e. g. UV-adhesives or two-component adhesives with heat treatment.

The upper wall of the observation field should have a thickness of approx. 0.17 mm. The indentation in the cover glass, the cross-section of the rods or the height of the microcell should be approx. 0.25 mm. The slide should have dimensions of 76 mm x 26 mm x 1 mm. The volume of the culture vessel should be approx. 1 ml. In order to facilitate working with the microscope, the vessel can be mounted so as to be inclined outwardly (Fig. 12).

The drawing of Fig. 1 shows the invention diagonally from above. Figs. 2 and 3 represent the cross-section of the observation field (6) in an embodiment as a slide (8) glued to a cover glass (9) with rods (11). Fig. 5 shows the embodiment as an indentation in a slide (8) with sealed (10) cover glass (9), and Fig. 6 shows the embodiment as microcell (3) on a slide.

The drawing of Figs. 7 and 8 shows a culture vessel with observation field in the embodiment of a threaded bottle (13) with flat capillary (3).

The drawing of Fig. 9 shows the embodiment of a culture vessel with observation field with a second bottle.

The drawing of Fig. 10 shows the arrangement for tests with microorganisms, where electric stimulations are made visible.

The drawing of Fig. 11 shows the manipulation of the liquid in the culture vessel, for example withdrawing, introducing or thoroughly mixing the culture liquid or reagents with a syringe through a septum (17).

The drawing of Fig. 12 shows the sucking off of the escaping liquid by means of a pipette as well as a culture vessel mounted in an inclined manner in order to facilitate working under a microscope.

The handling of the invention is described below:

1. Bring the culture vessel with observation field into an inclined position such that the end of the observation field faces upwards and remove the screwed plug (2).

2. Place liquid into the culture vessel (1) (13) by means of a pipette and bring it into a horizontal position for the observation field (6) to fill with liquid.

3. Seal the culture vessel. Now the observation field containing liquid with particles can be observed under a microscope. The liquid of the culture vessel can be microscoped in a flow by opening the plug (Fig. 12) (unscrew the screwed plug) and sucking off the liquid escaping at the end of the observation field. The escaping liquid can be sucked off with a pipette, a cotton bud or blotting paper. For examinations taking a relatively long time, the end of the observation field (7) can be temporarily or permanently sealed with Vaseline, silicone or putty.

4. In order to clean the used culture vessel with observation field, the seal (7) has to be removed. Then a cleaning solution (19) can be rinsed through the vessel under pressure with the syringe (20) by means of a cannula (21). Fig. 10, through the plug (2) containing a septum (17). With a scraper consisting of a small strip of thin plastic foil, particles adhering to the interior wall of the observation field (6) can be removed. Rinsing again with distilled water and drying the observation field (6) permits an air-bubble free filling of the observation field if it will be used again. Furthermore, there is the possibility of cleaning a culture vessel with observation field with removed plug (2) in an ultrasonic bath.

5. Liquids can be removed from or introduced into the culture solution by inserting the cannula (21), Fig. 10, of a syringe through the septum (17) into the culture vessel. By moving the piston (20), the liquid in the observation field (6) can be thoroughly mixed with the culture solution and with the liquids possibly introduced.

6. In order not to hit the culture vessel (1) with the objective during microscoping, a marking (18) can be attached to the observation field (e.g. with a red glass writing pencil)

7. If several objectives are worked with and the rotating nosepiece is to be rotated, one has to remember the site at the vernier of the mechanical stage in order to find the site again after having left it and changed the objective.

8. The culture vessel with observation field can be handled as sterile closed system when it is correspondingly sealed (7) and it can also be used for cultivating or conserving anaerobic microorganisms for a relatively long time. Furthermore, external influences, e.g. conservation in refrigerators or incubators or radiological manipulations, can be examined.

In a particular embodiment of the invention, conductors are attached and the reaction to electrical stimulations with protozoans is made visible. In the drawing (Fig. 10), one embodiment is shown.

The culture vessel (1) with observation field (6) is attached to a slide (8), filled with a paramecium culture solution and sealed at the end of the observation field with Vaseline (7). The conductors (15 + 16) introduced into the liquid are connected at the terminals with a dc power source having an amperage of approx. 6V and the polarity of which can be reversed. Under a microscope, in the observation field (6) of the invention, the paramecia can be seen swimming to the negative terminal in the electric field. If the polarity is reversed, the protozoans immediately return and swim again towards the cathode.

Another phenomenon easy to handle is the phototactic reaction of light-seeking green algae. The culture vessel with observation field is filled with a green algae culture solution (e.g. Euglena). In the path of light rays of the microscope, an agglomeration of the green algae can

be seen in the observation field (6) after a short time. If the light source is switched off and on again, the algae appear again in the field of vision.

Furthermore, the invention can be handled as ready culture stock (some microorganisms form cysts when they dry up in which they survive for a long time in a sleep-like state). In favourable conditions, they awake to normal life with locomotion, ingestion and reproduction. The culture vessel can contain nutrients with cysts in a dry state. The filling of the culture vessel with water suffices for activating the vital operations.

In another embodiment of the invention, a second culture vessel is attached to the slide at the end of the observation field, Fig. 9. The microscopic examination facility of flowing liquids in the observation field is described as follows:

1. The left culture vessel is made airtight with a screwed plug.

2. The right culture vessel is open and is filled with liquid.

3. Under the microscope, by unscrewing the left plug, air escapes and makes half of the liquid flow through the observation field (6). This operation can be stopped at any time by screwing the plug.

4. Sucking off the liquid in the left vessel with a pipette causes a flow of the remaining liquid through the observation field.

Finally, the culture vessel with observation field can also be used for microscoping small inorganic particles contained in a liquid.

List of reference numerals

1 Culture vessel (threaded bottle with removed bottom)
2 Plug (plastic screwed plug)
3 Flat capillary
4 Permanently sealing conglutination (e.g. UV-adhesive or two-component adhesive)
5 Open connection to the observation field
6 Observation field
7 Temporary or permanent sealing (e.g. with Vaseline, putty or silicone)
8 Slide
9 Cover glass
10 Permanently sealing conglutination
11 Round or flat rods glued so as to provide permanent sealing
12 Grinding surface of the threaded bottle
13 Threaded bottle with ground off bottom
14 Permanently connecting sealing between threaded bottle and flat capillary
15 Conductor (anode)
16 Conductor (cathode)
17 Septum (screwed plug insert)
18 Marking (red foil, or attached with glass writing pencil)
19 Liquid to be incorporated or removed (e.g. culture liquid, reagents or cleaning fluid)
20 Syringe
21 Cannula
22 Liquid with particles
23 Pipette (for sucking off the escaping liquid)

Patent Claims

1. Culture vessel with observation field, **characterized by** a vessel (1) with open connection (5) to an observation field (6) for microscopic examinations according to Fig. 1, Fig. 1 being part of the claim.

2. Culture vessel with observation field according to claim 1, characterized in that a thin-walled observation field (6) for microscopic examinations formed by a slide (8) with cover glass (9) and conglutinated rods (11), is permanently connected by gluing with a thick-walled tube (1) or culture vessel, thereby providing a seal (4) at the front side, the observation field (6) remaining open inside (5).

3. Culture vessel with observation field according to claim 1, characterized in that the observation field (6) can be formed as an indentation in a slide (8) with a conglutinated (10) cover glass (9).

4. Culture vessel with observation field according to claim 1, characterized in that the observation field (6) can be formed as microcell (3).

5. Culture vessel with observation field according to claim 4, characterized in that a microcell (3) can be introduced through a vessel (13) ground open at the bottom (12) and be permanently connected by gluing, thus providing a sealing (4) outside, the observation field (6) remaining open (5) inside.

6. Culture vessel with observation field according to one of claims 2 to 4, characterized in that the observation field (6) can be sealed at the end.

7. Culture vessel with observation field according to claim 6, characterized in that at the end of the observation field, a sealed conductor (16) can be attached (Fig. 10). Fig. 10 being part of the claim.

8. Culture vessel with observation field according to claim 1, characterized in that the vessel (1) can be sealed.

9. Culture vessel with observation field according to claim 8, characterized in that pipettes or hollow needles can be put through the plug (2) and liquids can be introduced into the culture vessel (1) and (13) (Fig. 10). Fig. 10 being part of the claim.

10. Culture vessel with observation field according to one of claims 1 to 9, characterized in that at the end of the observation field, a second sealable vessel with an open connection (5) to the observation field (6) can be attached (Fig. 9). Fig. 9 being part of the claim.